REMARKS

I. Status of the Claims

Claims 50, 58, 61, and 63 are amended. Claims 1-49, 51-52, 54-56, and 64-72 are cancelled without prejudice for filing in a continuation application. Claims 50, 53, and 57-63 are pending.

Support for amended claim language is found throughout the specification and claims as originally filed. Support for "wherein the lysing solution comprises a chaotropic agent" is found, for example, at page 7, lines 6-19, and at all locations where lysis buffer is used in the working examples since the lysis buffer contains 4 M GuSCN.

Support for "wherein small RNA molecules flow through the solid support and large RNA molecules bind to the solid support" is found, for example, at page 10, line 28, to page 11, line 1, at Example 3, page 32, line 12 to page 33, line 16 and at FIG. 7 where large mRNA is completely removed from small RNA as demonstrated by the partitioning to a second column.

Support for "an alcohol concentration of at least about 55%" is found, for example, at Example 1, page 30, lines 1 and 17; at Example 3, page 32, line 26; at Example 6, page 36, line 7; at Example 7, page 36, line 17, and at Example 8, page 37, line 11.

Support for "wherein small RNA molecules bind to the second solid support" is found, for example, at page 10, line 29, to page 11, line 1, at Example 3, page 33, lines 12-16 and at FIG. 7 where large mRNA is completely removed from small RNA as demonstrated by the partitioning to a second column.

Support for "wherein small RNA molecules have 200 nucleotides or fewer, and large RNA molecules have greater than 200 nucleotides" is found at page 6, line 14, at Example 3 and at FIG. 7 which demonstrates separation of U2 small RNA (187 nt) and let-7 small RNA (22 nt) from GAPDH mRNA and from β-actin mRNA.

Further amendments address informalities and proper antecedent bases. Applicant submits that no new matter has been introduced by the amended claims.

II. Rejections of Claims 4 and 5 under 35 U.S.C. §112, Second Paragraph

The Action states a rejection of Claims 4 and 5 under 35 U.S.C. §112, second paragraph, for indefiniteness regarding "using 4 volumes of ethanol." Office Action pages 5-6.

Response

Applicant respectfully traverses this rejection. However, in order to expedite prosecution and simplify issues for appeal, Claims 4 and 5 are canceled without prejudice for filing in a continuation application. Applicant therefore respectfully requests withdrawal of the rejection of Claims 4 and 5 under 35 U.S.C. §112, second paragraph.

III. Rejection of Claims 1-17, 19-48, 50 and 53-72 Under 35 U.S.C. §103

Claims 1-17, 19-48, 50 and 53-72 are rejected as being unpatentable over a manual for micro RNA isolation (Strategene, 2000) in view of Bost *et al.* (U.S. 6,111,096 (sic)U.S. Patent Publication No. 2003/0138828 ('828)), in further view of Ekenberg *et al.* (U.S. Patent No. 6,218,531 ('531)). See Office Action at pages 7-14.

Response

Applicant assumes that this rejection intends to cite Bost (U.S. Patent Publication No. 2003/0138828) as cited in the PTO-892 form mailed with the Office Action of 08/23/07. U.S. Patent No. 6,111,096 is to Laugharn, Jr. *et al.,* not to Bost *et al.*. Applicant respectfully requests that the examiner clarify this rejection.

Applicant respectfully traverses this rejection. Claims 1-49, 51-52, 54-56, and 64-72 are canceled without prejudice for filing in a continuation application in order to simplify issues for prosecution and appeal. Pending Independent Claim 50 and claims dependent thereof set forth the invention as described, for example, by working Example 3 and the data of FIG. 7.

In *KSR International Co. v. Teleflex Inc.* 127 S. Ct. 1727, April 30, 2007, the Supreme Court reiterated that the framework for determining obviousness under §103 it had set out in *Graham v. John Deere Co.,* 383 U.S. 1, 148 USPQ 459 (1966) continues to "define the inquiry that controls" determination of obviousness or nonobviousness of the claimed subject matter. As set forth in *Graham*, obviousness under 35 U.S.C. §103 is a question of law based on factual

inquiries: (1) the scope and the content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; and (4) objective evidence of secondary considerations. In *KSR*, the Supreme Court cited that secondary considerations might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented.

In addition, the *KSR* decision states that a rejection on obviousness grounds must identify "an apparent reason to combine the known elements *in the fashion claimed by the patent at issue*. To facilitate review, this analysis should be made explicit." *KSR Int'l. Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (emphasis added) (citing *In re Kahn*, 441, F.3d 977, 988 (Fed. Cir. 2006)).

Stratagene's Micro RNA isolation manual in view of Bost *et al.* in further view of Ekenberg *et al.*: As stated by the Office Action, the Stratagene manual does not teach the use of solid support or eluting small RNA from the solid support.

Further, the Stratagene manual does not teach or suggest the following elements of the invention as set forth by Claim 50: c) adding to the lysate an alcohol solution to form a lysate/alcohol mixture of about 20% to about 35% alcohol; d) applying the lysate/alcohol mixture of about 20% to about 35% alcohol to a first solid support wherein small RNA molecules flow through the solid support and large RNA molecules bind to the solid support; e) collecting flow-through lysate/alcohol mixture containing the small RNA molecules; f) adding to the flow-through lysate/alcohol mixture an alcohol solution to an alcohol concentration of at least about 55%; g) applying the lysate/alcohol mixture of at least about 55% to a second solid support wherein small RNA molecules bind to the second solid support; and h) eluting small RNA molecules from the second solid support wherein small RNA molecules have 200 nucleotides or fewer, and large RNA molecules have greater than 200 nucleotides.

At page 1 of the Stratagene manual, the fourth full paragraph, the Micro RNA Isolation Kit is described as "a perfect system for extracting RNA from *very small samples*." The next sentence states: "When biological *samples are present in small amounts* ... ." Therefore, the "micro" portion of the title of this manual refers to small sized samples, not isolation of RNA of small size. That the focus of this manual is RNA isolation from small samples is further supported by the information at page 12 on the formaldehyde gel protocol which recites the size

range of 400-2000 bases for most mRNA. Clearly, the manual does not contemplate isolation of small RNA molecules less than 400 bases.

Bost *et al.*, at Example 13, cite isolation of DNA and the ability of high concentrations of RNA to inhibit binding of genomic DNA to silicon dioxide particles. No alcohol was used during binding. At Figure 13, the recovery of nucleic acid was visualized using 1% agarose gel electrophoresis. The smallest-sized band of the "Input" lanes (that may correspond to small RNA) appears not recoverable by either of the pH 6 or the pH 10 extractions under any of the RNA/DNA ratios. Similarly, Example 14 cites binding of RNA and DNA to silicon dioxide in the absence of alcohol. Figure 14 shows recovery of nucleic acid on a 0.8% agarose gel. No small RNA molecules appear to be present. Therefore, small RNA molecules are not isolated by the Bost *et al.* procedures.

Therefore, the Stratagene manual in view of Bost *et al.* do not teach or suggest the following elements of the invention as set forth by Claim 50: c) adding to the lysate an alcohol solution to form a lysate/alcohol mixture of about 20% to about 35% alcohol; d) applying the lysate/alcohol mixture of about 20% to about 35% alcohol to a first solid support wherein small RNA molecules flow through the solid support and large RNA molecules bind to the solid support; e) collecting flow-through lysate/alcohol mixture containing the small RNA molecules; f) adding to the flow-through lysate/alcohol mixture an alcohol solution to an alcohol concentration of at least about 55%; g) applying the lysate/alcohol mixture of at least about 55% to a second solid support wherein small RNA molecules bind to the second solid support; and h) eluting small RNA molecules from the second solid support wherein small RNA molecules have 200 nucleotides or fewer, and large RNA molecules have greater than 200 nucleotides.

Ekenberg *et al.* lose small RNA molecules at the step of discarding the contents of the collection tube at lines 38-39 of column 18. Small RNA molecules are essentially not bound under the conditions of steps, 3, 4, and 5 of lines 17-28. Since the contents of the collection tube are discarded, there is no small RNA to obtain from the rest of the Ekenberg *et al.* procedure. For any incidental small RNA that may have bound, it is removed by washing with 2.19 M GTC at line 54 of column 18 and by subsequent washes with 60% ethanol. That the Ekenberg *et al.*

patent is focused on yield and purity (rather than size of RNA isolated) is further evidenced by Tables 1-5 that provide such data.

Bost *et al.* in combination with Ekenberg *et al.* do not remove the deficiencies of the Stratagene manual since none of the references, alone or in combination, or in light of one of ordinary skill in the art, teach, suggest or cite procedures that achieve isolation of small RNA molecules away from large RNA molecules as set forth by independent Claim 50.

Difference between the prior art and the claims at issue

Some of the differences between the combination of cited prior art and the claims at issue include a method for isolating small RNA molecules from a sample containing cells, the method comprising: a) lysing the cells in a lysing solution to produce a lysate; b) extracting RNA molecules from the lysate with an extraction solution comprising phenol; c) adding to the lysate an alcohol solution to form a lysate/alcohol mixture of about 20% to about 35% alcohol; d) applying the lysate/alcohol mixture of about 20% to about 35% alcohol to a first solid support wherein small RNA molecules flow through the solid support and large RNA molecules bind to the solid support; e) collecting flow-through lysate/alcohol mixture containing the small RNA molecules; f) adding to the flow-through lysate/alcohol mixture an alcohol solution to an alcohol concentration of at least about 55%; g) applying the lysate/alcohol mixture of at least about 55% to a second solid support wherein small RNA molecules bind to the second solid support; and h) eluting small RNA molecules from the second solid support wherein small RNA molecules have 200 nucleotides or fewer, and large RNA molecules have greater than 200 nucleotides.

Applicants submit that the combination of Stratagene's Micro RNA isolation manual in view of Bost *et al.* further in view of Ekenberg *et al.* does not render the subject matter set forth by the pending claims obvious, in part, because the combination of prior art references fails the "objective reach of the claims" test regarding independent Claim 50.

The *KSR* decision held that when there is a market pressure to solve a problem and there are a finite number of identified, *predictable* solutions, a person of ordinary skill has a good reason to pursue the known options within his or her technical grasp (*KSR*, see section II(c)). The courts have long recognized that life science inventions are less predictable than in other

areas. Here, the combination of references cited against the claims lacks a teaching of any solutions, let alone a predictable solution to the problem of isolation of small RNA molecules.

For the above cited reasons, Applicant submits that the invention as set forth by the independent Claim 50 is patentable under U.S.C. §103 and respectfully requests that the rejection be withdrawn. An essential characteristic of a proper dependent claim is that it shall include every limitation of the claim from which it depends. Therefore, a dependent claim is allowable when the claim from which it depends is allowable. Claims 53 and 57-63 are directly or indirectly dependent upon Claim 50. Therefore, Applicants submit that said claims are patentable also and respectfully request that the rejection under U.S.C. §103 be withdrawn.

IV. Conclusion

Applicant believes that the present document is a full and complete response to the Action dated March 10, 2008. Applicant believes that the application is in condition for allowance and respectfully requests issuance of a Notice of Allowance. If the Examiner does not consider the application to be in condition for allowance, Applicant requests that he call the undersigned at (512)721.3654 to set up an interview.

Respectfully submitted,

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